# SEASONAL CHANGES IN THE PROPERTIES OF FROG End-Plate Channels

CAROL A. LEWIS
*Department of Biophysical Sciences, State University of New York at Buffalo, Buffalo, New York 14214*

ABSTRACT The properties of the acetylcholine (ACh)-activated channel at the frog neuromuscular junction were studied using a two-microelectrode voltage clamp. The reversal potential was determined by interpolation of the ACh-induced current vs. voltage relation, while the single-channel conductance and the mean channel lifetime were calculated from fluctuation analysis of the mean ACh-induced end-plate current. Seasonal changes were observed in some of the measured parameters. While the reversal potential and the mean channel lifetime remained constant throughout the year, the single-channel conductance did not. The single-channel conductance was on the average 35% higher in the winter than in the summer. This effect could have survival value for hibernating frogs.

Seasonal changes in the physiology of amphibians such as in the distribution of frog extrajunctional acetylcholine (ACh) receptors (Feltz and Mallart, 1971; Dreyer and Peper, 1974) and in the passive electrical properties of toad muscle membrane (Dulhunty and Gage, 1973) have been observed. While performing a series of voltage-clamp experiments on the properties of the ACh-activated channel at the frog neuromuscular junction, I observed seasonal changes in some of the measured parameters. While the reversal potential ($V_o$) and the mean channel lifetime ($\tau$) remained constant throughout the year, the single-channel conductance ($\gamma$) did not. The single-channel conductance was on the average 35% higher in the winter than in the summer. This effect could have survival value for hibernating frogs.

The experiments described in this report were performed from August 1982 through February 1983. The frogs were obtained from Nasco (Fort Atkinson, WI). In each shipment, half of the frogs were kept at room temperature and half were kept in a refrigerator at ~8°C. No differences were observed in the results from the frogs kept at room temperature from those kept in the refrigerator; the results were therefore all averaged together to give the mean values reported here. The frogs were used within a month of their arrival, and during this time they were not fed. Muscles from frogs obtained from the supplier from August 1982 through November 1982 gave similar values for the single-channel conductance, and these frogs are called summer frogs in this report. All the frogs obtained from December 1982 through February 1983 gave similar higher values for the single-channel conductance and are called winter frogs.

The experimental procedure has been previously described (Lewis, 1979). The cutaneous pectoris muscle was removed from northern *Rana pipiens* and dissected down to a monolayer of muscle fibers. A two-microelectrode voltage clamp was used, and a third microelectrode filled with ~3 M acetylcholine ACh was used for iontophoretic application of ACh to the end-plate. The temperature was maintained with a Peltier device in the microscope stage, and experiments were performed at 8, 12, 16, and 20°C. The reversal potential was calculated from interpolation of the ACh-induced end-plate current (EPC) vs. voltage relationship. The ACh-induced EPC current was observed for holding potentials of −90, −70, and −50 mV and stored on an FM tape recorder. The current data were bandpass filtered at 1–400 Hz, sampled at 1 kHz, and analyzed using a data analyzer (Nic Med-80; Nicolet Instrument Co., Madison, WI). Single time constant Lorentzians were fit to the difference power spectra by eye. The mean channel lifetime was calculated from the cutoff frequency, $f_c$, using the equation: $\tau = 1/(2\pi f_c)$. The cutoff frequency is assumed to be normally distributed so that the SEM can be calculated. The stated error limits on the mean channel lifetime were calculated from the reciprocals of ($f_c$ + SEM) and ($f_c$ − SEM).

The reversal potential for end-plate channels was similar in both summer and winter frogs. For example, at 12°C, the mean reversal potential was −6.0 ± 0.4 mV (± SEM) ($n$ = 21) for summer frogs vs. −6.2 ± 0.5 mV ($n$ = 12) for winter frogs. Similarly, there were no significant differences between the mean channel lifetimes for summer and winter frogs. For example, the mean channel lifetimes at 12°C and for holding potentials of −90, −70, and −50

mV were for summer frogs 5.77 + 0.42 − 0.36 ms (± error limits as described in the preceding paragraph) ($n$ = 4), 3.46 ± 0.07 ms ($n$ = 21), 3.06 + 0.28 −0.23 ms ($n$ = 4), and for winter frogs 5.17 + 0.40 −0.34 ms ($n$ = 5), 3.55 ± 0.11 ms ($n$ = 6), and 3.05 + 0.19 −0.17 ms ($n$ = 5), respectively.

The single-channel conductance measured in winter frogs was larger than the value measured in summer frogs. As an example, the average single-channel conductance measured at a holding potential of −70 mV and a temperature of 12°C from August through November 1982 was 28.3 ± 0.7 pS (± SEM) ($n$ = 21) and was 38.4 ± 1.3 pS ($n$ = 6) when measured from December 1982 through February 1983. Table I gives a comparison of the $\gamma$ values measured in summer frogs vs. the $\gamma$ values measured in winter frogs in the form of the ratio $\gamma$ winter/$\gamma$ summer. There was no discernable trend in the value of the ratio with respect to the holding potential or temperature; therefore, all twelve values were averaged together to give a mean value for the ratio of 1.35 ± 0.15 (± SD).

The single-channel conductance and the mean channel lifetime are a function of temperature in both summer and winter frogs. Fig. 1 shows a plot of log $\gamma$ vs. $1/T$ for the various holding potentials, while Fig. 2 shows a plot of log $\alpha$ (where $\alpha = 1/\tau$) vs. $1/T$ for a holding potential of −70 mV. The lines drawn through the data points are a linear least-squares fit. The $Q_{10}$ values for $\gamma$ and $\alpha$ were calculated from the slopes of the lines and the results are listed in Table II. It appears as though $\gamma$ is slightly less temperature dependent in winter frogs. If the $Q_{10}(\gamma)$ values for the various holding potentials are averaged together, then the $Q_{10}(\gamma)$ for summer frogs is 1.58 ± 0.07 (± SD), whereas it is 1.46 ± 0.04 for winter frogs. There is no consistent difference in the temperature dependence of the mean channel lifetime for summer and winter frogs.

The properties of the ACh-activated channel in winter and in summer frogs can be analyzed in terms of an Eyring rate theory model of the channel (Lewis and Stevens, 1979). The fact that the reversal potential is approximately the same throughout the year indicates that the difference in the barrier heights for Na relative to K remains constant. The increase in the single-channel conductance in winter, then, would result if the barriers for Na and K ions both decreased slightly by the same amount. This difference in barrier height could possibly be due to seasonal changes in the lipid environment of the end-plate channel.

Other investigators have noted some seasonal changes in amphibian physiology. Seasonal changes have been

TABLE I
COMPARISON OF SINGLE-CHANNEL CONDUCTANCE VALUES MEASURED IN SUMMER VS. IN WINTER FROGS

| Temperature | $\gamma$ Winter/$\gamma$ Summer | | |
|---|---|---|---|
| | −90 mV* | −70 mV* | −50 mV* |
| 8°C | 1.51 ± 0.21‡ | 1.23 ± 0.20‡ | 1.49 ± 0.58‡ |
| 12°C | 1.14 ± 0.27 | 1.36 ± 0.18 | 1.52 ± 0.51 |
| 16°C | 1.59 ± 0.26 | 1.20 ± 0.16 | 1.37 ± 0.20 |
| 20°C | 1.17 ± 0.15 | 1.29 ± 0.20 | 1.29 ± 0.20 |

The single-channel conductance is calculated from the zero frequency asymptote [$S(0)$] from the following equation: $\gamma = S(0)\pi f_c / [u_1(V - V_0)]$, where $u_1$ is the mean end-plate current and $f_c$ is the cutoff frequency.
*Holding potential.
‡±SD.

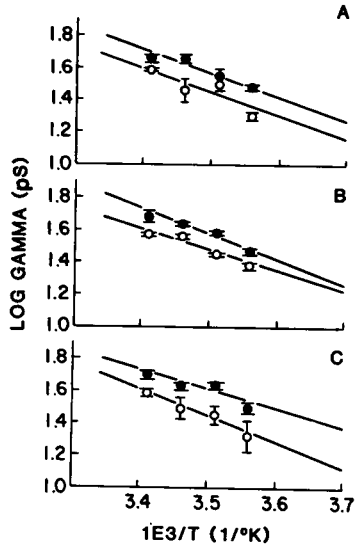


FIGURE 1 A plot of log $\gamma$ vs. $1/T$. The error bars indicate ± 1 SEM. The straight line (—) is a linear least-squares fit to the data points. The open symbols (o) indicate data for summer frogs, whereas the filled symbols (•) are for winter frogs. The graphs are for data at the following holding potentials: ($A$) −90 mV; ($B$) −70 mV; ($C$) −50 mV.

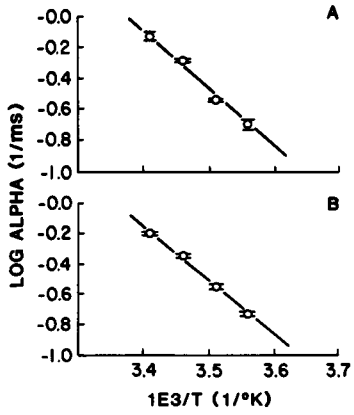


FIGURE 2 A plot of log $\alpha$ vs. $1/T$ (where $\alpha = 1/\tau$) for a holding potential of −70 mV. The error bars indicate ± 1 SEM. The straight line (—) is a linear least-squares fit to the data points. Data for ($A$) summer frogs and ($B$) winter frogs.

TABLE II
COMPARISON OF $Q_{10}$ VALUES FOR SUMMER AND WINTER FROGS

| Holding potential | $Q_{10}$ ($\gamma$) | | $Q_{10}$ ($\tau$) | |
|---|---|---|---|---|
| | Summer | Winter | Summer | Winter |
| *mV* | | | | |
| −90 | 1.61 ± 0.09* | 1.45 ± 0.09 | 3.44 ± 0.09 | 3.04 ± 0.11 |
| −70 | 1.50 ± 0.05 | 1.51 ± 0.08 | 3.21 ± 0.18 | 2.95 ± 0.10 |
| −50 | 1.64 ± 0.24 | 1.43 ± 0.11 | 2.91 ± 0.31 | 3.42 ± 0.18 |

The mean channel lifetime, $\tau$, is calculated from the cutoff frequency ($f_c$) of the Lorentzian fitted to the spectral density according to the following equation: $\tau = 1/(2\pi f_c)$.
*±SD.

observed in the extrajunctional sensitivity of frog muscles to ACh. Feltz and Mallart (1971) found that the extrajunctional sensitivity was highest in the winter, whereas Dreyer and Peper (1974) observed the maximum extrajunctional sensitivity in the summertime. Nayler (1957) found evidence of a possible seasonal change in the permeability of cardiac cell membrane of the toad *Bufo marinus* in that the hibernating heart showed a decreased sensitivity to the cardiac glycoside strophanthin-G. Dulhunty and Gage (1973) observed seasonal changes in the electrical properties of toad sartorius muscle fibers. They found that the space constant was 70% larger in summer, the internal resistivity was 32% higher in the winter, the specific membrane resistance was 95% higher in the summer, and the specific membrane capacitance was 68% higher in the winter. They suggest that one possible explanation for some of these changes could be differences in the degree of saturation of membrane lipid with season. Penefsky et al. (1981) observed seasonal variations in the electrical responses of toad cardiac muscle. The resting potential was slightly hyperpolarized in the winter, and, while the amplitude of the action potential remained the same, the overshoot was smaller and the duration was longer during the winter.

The observed variation in single-channel conductance is not an artifact due to changes in the electrical properties of the muscle fibers as observed by Dulhunty and Gage (1973). The single-channel conductance would be underestimated if the voltage clamp were inadequate. Dulhunty and Gage (1973) observed that the space constant was shorter during the winter, and a shorter space constant could result in an inadequate voltage clamp. This explanation would result in lower values being calculated for $\gamma$ in the winter, while the opposite is actually observed.

One artifact that would result in a lower apparent value for the single-channel conductance in the summer is a higher incidence of unresolved flickering of the channel during the summer. From the experimental data, it is possible to calculate limits on the rate of flickering. The power spectral density plots show no apparent sign of a second component out to the filter cutoff frequency. For unresolved flickering to account for the decreased $\gamma$ during the summer, the $S(0)$ for the second component would have to be $<10^{-23}$ $A^2$ s, a typical value for $S(400)$. The corresponding value for the cutoff frequency for the second component must be >2 kHz for the variance of the second component to be 35% of the variance of the first component. If the assumption is made that the channel does not flicker during the winter (i.e., $P[0] = 1$), then the observed decrease in the single-channel conductance during the summer would correspond to a probability of the channel being open of $P(0) = 0.74$. If a further assumption is made that the channel opening and closing is a two-state process, then the rate constants for channel opening ($k_o$) and channel closing ($k_c$) are related to the time constant for the process ($\tau$) and to the probability that the channel is open ($P[0]$) by the following equations: $\tau = 1/(k_o + k_c)$ and $P(0) = k_o/(k_o + k_c)$. When the two simultaneous equations are solved for the rate constants, the result is that $k_o = 9 \times 10^3$/s and $k_c = 3.3 \times 10^3$/s. Consequently, the observed reduction in the single-channel conductance during the summer would result if every 300 $\mu$s a channel closed for ~100 $\mu$s. These numbers are plausible considering the flickering rates that have been observed for other ACh-activated channels (Auerbach and Sachs, 1984). (The above calculation was done using an arbitrary value for $S(0)$ of $10^{-23}$ $A^2$ s. Using a smaller value for $S(0)$ would result in calculating a larger value for $f_c$ to produce the same variance and the same decrease in $\gamma$. This change would result in even larger values being calculated for the opening and closing rate constants). However, a consideration of the observed temperature dependence of $\gamma$ tends to rule out the possibility of significant flickering during the summer. Unresolved flickering would be expected to result in the single-channel conductance showing a large temperature dependence since it is likely that the relevant rate constants are highly temperature dependent. The observed $Q_{10}$ values of 1.4 to 1.6 are approximately what would be expected for a process governed by diffusion (Robinson and Stokes, 1959). In summary, the observed variation in $\gamma$ with season is not due to the changed electrical properties of the muscle fibers and is probably not due to unresolved flickering during the summer.

The fact that the single-channel conductance is higher in winter frogs compared with summer frogs could have survival value for hibernating frogs. This would result in a larger EPC when the nerve terminal is activated at the lower temperatures encountered in the wintertime. This adaptation during the winter could increase the safety margin for neuromuscular transmission.

The effect reported in this paper is a difference in the single-channel conductance in winter and and in summer frogs. The cause of this effect cannot be assigned with certainty to any particular factor. The change in single-channel conductance is not necessarily a chronobiologic

effect. One possible cause is the seasonal change in metabolism that occurs in frogs; carbohydrate catabolism is predominate during the summer, and the utilization of stored glucogen and fat predominates during the winter (Smith, 1950; Nayler, 1957; Mizell, 1965). The difference in metabolism may alter the lipid environment of the end-plate channel such that the single-channel conductance is affected.

Another possible explanation is that there may be genetic differences in the energy profile through the channel such that the single-channel conductance is changed while the reversal potential and the mean channel lifetime remain the same. The frogs were obtained from a single supplier (Nasco) and are collected from lake regions in North Dakota, Wisconsin, and Minnesota. Frogs from these regions are collected at various times throughout the year; the possibility exists therefore that all shipments of frogs received from November 1982 were from one particular region. If there are genetic differences in the structure of the end-plate channel, then this could explain some of the conflicting results in the literature on end-plate channel properties even with studies using the same biological preparation, *Rana pipiens*.

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